Antibody Compositions for Preparing Enriched Human Hematopoietic and Tumor Cell Preparations

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Patent Number: CA2191655
Publication date: 1997-06-02
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Requested Patent: ☐ CA2191655
Application Number: CA19962191655 19961129
Priority Number(s): US19950566295 19951201
IPC Classification: C12N5/08 ; C07K16/28
EC Classification:
Equivalents:

Abstract

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OPIC
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CIPO
Canadian Intellectual Property Office

(21) (A1) 2,191,655
(22) 1996/11/29
(43) 1997/06/02

(51) Int.Cl.[6] C12N 5/08; C07K 16/28

(19) (CA) **APPLICATION FOR CANADIAN PATENT** (12)

(54) Antibody Compositions for Preparing Enriched Human Hematopoietic and Tumor Cell Preparations

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(30) (US) 08/566,295 1995/12/01

(57) 32 Claims

Notice: This application is as filed and may therefore contain an incomplete specification.

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ABSTRACT OF THE DISCLOSURE

The present invention relates to an antibody composition which contains antibodies specific for glycophorin A, CD3, CD24, CD16, CD14, and optionally CD45RA, CD38, CD36, CD38, CD56, CD2, CD19, CD66e, CD66b, and antibodies specific for antigens expressed on non-hematopoietic tumor cells. A process is provided for enriching and recovering human hematopoietic progenitor cells and stem cells in a sample containing human hematopoietic differentiated, progenitor, and stem cells, and tumor cells. The invention also contemplates tumor-enriching antibody compositions containing antibodies to glycophorin A, CD3, CD19, CD36, CD14, CD16, CD66b, CD66e, CD38, and optionally CD45, CD41, CD33, CD20. CD22, CD29, CD2, CD45RA, and/or CD10 and a process for enriching for metastatic tumor cells in a sample containing the tumor cells and hematopoietic cells. The invention also relates to kits for carrying out these processes and to the cell preparations prepared by the processes.

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Title: Novel Antibody Compositions for Preparing Enriched Human Hematopoietic and Tumor Cell Preparations

FIELD OF THE INVENTION

The present invention relates to novel antibody compositions, and processes and kits for preparing cell preparations enriched for specific cell types and the use of the cell preparations. The invention also relates to purified cell preparations.

BACKGROUND OF THE INVENTION

Blood cells have a relatively short life span and need to be replenished throughout life. In adults, blood cell formation or hematopoiesis takes place in the bone marrow, but blood-forming stem cells can also be found in peripheral blood. Hematopoietic cells represent a hierarchy of proliferating and differentiating cells. The most abundant are the differentiating or lineage committed cells. These cells have limited or no proliferative capacity and represent specialized end cells that are found in blood, and their immediate precursors.

The immediate precursors of the differentiating cells are the progenitor cells. Most of these cells are restricted to differentiate along a single lineage but they may have quite extensive proliferative capacity. Progenitor cells appear morphologically as blast cells, and they typically do not have specific features of the hematopoietic lineage to which they are committed.

Progenitor cells are derived from stem cells. Stem cells have been historically defined by their ability to self-renew as well as to generate daughter cells of any of the hematopoietic lineages. The presence of stem and progenitor cells may be detected by their ability to produce colony-forming cells in culture. They may also be detected by screening for the CD34 antigen which is a positive marker for early hematopoietic cells including colony forming cells and stem cells. At present, the long term culture initiating cell (LTCIC) assay appears to be the best way to detect stem

cells, or at least the most primitive progenitor cells, using tissue culture methodologies.

There is a continued interest in developing stem cell purification techniques. Pure populations of stem cells will facilitate studies of hematopoiesis. Transplantation of hematopoietic cells from peripheral blood and/or bone marrow is also increasingly used in combination with high-dose chemo- and/or radiotherapy for the treatment of a variety of disorders including malignant, nonmalignant and genetic disorders. Very few cells in such transplants are capable of long-term hematopoietic reconstitution, and thus there is a strong stimulus to develop techniques for purification of hematopoietic stem cells. Furthermore, serious complications and indeed the success of a transplant procedure is to a large degree dependent on the effectiveness of the procedures that are used for the removal of cells in the transplant that pose a risk to the transplant recipient. Such cells include T lymphocytes that are responsible for graft versus host disease (GVHD) in allogenic grafts, and tumor cells in autologous transplants that may cause recurrence of the malignant growth. It is also important to debulk the graft by removing unnecessary cells and thus reducing the volume of cyropreservant to be infused.

Hematopoietic cells have been separated on the basis of physical characteristics such as density and on the basis of susceptibility to certain pharmacological agents which kill cycling cells. The advent of monoclonal antibodies against cell surface antigens has greatly expanded the potential to distinguish and separate distinct cell types. There are two basic approaches to separating cell populations from bone marrow and peripheral blood using monoclonal antibodies. They differ in whether it is the desired or undesired cells which are distinguished/labeled with the antibody(s).

In positive selection techniques the desired cells are labeled with antibodies and removed from the remaining unlabeled/unwanted cells. In negative selection, the unwanted cells are labeled and removed. Antibody/complement treatment and the use of immunotoxins are

negative selection techniques, but FACS sorting and most batch wise immunoadsorption techniques can be adapted to both positive and negative selection. In immunoadsorption techniques cells are selected with monoclonal antibodies and preferentially bound to a surface which can be removed from the remainder of the cells e.g. column of beads, flasks, magnetic particles. Immunoadsorption techniques have won favour clinically and in research because they maintain the high specificity of targeting cells with monoclonal antibodies, but unlike FACSorting, they can be scaled up to deal directly with the large numbers of cells in a clinical harvest and they avoid the dangers of using cytotoxic reagents such as immunotoxins, and complement.

Current positive selection techniques for the purification of hematopoietic stem cells target and isolate cells which express CD34 (approximately 1-2% of normal bone marrow) (Civin, C.I., Trischmann, T.M., Fackler, M.J., Bernstein, I. D., Buhring, H.J., Campos, L. et al. (1989) Report on the CD34 cluster workshop, In: Leucocyte typing IV, White Cell Differentiation Antigens. Knapp, W., Dorken, B., Gilks, W.R., Reiber, E P., Schmidt, R. E., Stein, H., and Kr. von den Borne, AE.G Eds., Oxford University Press. Oxford, pp.818). Thus, the potential enrichment of hematopoietic stem cells using this marker alone is approximately 50 fold. Available techniques typically recover 30-70% of the $CD34^+$ cells in the start suspension and produce an enriched suspension which is 50-80% $CD34^+$ (Ishizawa, L. et al., In: Hematopoietic Stem Cells: The Mulhouse Manual eds. Wunder, E., Sovalat, H. Henon, P., and Serke, S. AlphaMed Presa, Ohio pp171-182; Shpall, E.J., et al. (1994), J. of Clinical Oncology 12:28-36; Winslow, J.M., et al. (1994), Bone Marrow Transplantation 14:265-271; Thomas, T.E., (1994), Cancer Research, Therapy and Control 4(2): 119-128). The positive selection procedures suffer from many disadvantages including the presence of materials such as antibodies and/or magnetic beads on the $CD34^+$ cells, and damage to the cells resulting from the removal of these materials.

Negative selection has been used to remove minor populations of cells from clinical grafts. These cells are either T-cells or tumor cells that pose a risk to the transplant recipient. The efficiency of these purges varies with the technique and depends on the type and number of antibodies used. Typically, the end product is very similar to the start suspension, missing only the tumor cells or T-cells.

Transplants of purified stem cells without differentiated or lineage committed cells will give short and long-term hematopoietic support (Shpall, E.J., et al. (1994), J. of Clinical Oncology 12:28-36). Since differentiated cells make up a vast majority of the cells in bone marrow and blood, depletion of these cells produces a much smaller cell suspension. The number of cells in the final product and the degree of enrichment of progenitor/stem cells will depend on the efficiency of the antibody targeting and the removal of labeled cells.

There are several studies that enrich for hematopoietic stem cells by depleting lineage committed cells but all require a number of positive or negative selection steps to achieve the desired degree of enrichment (50 fold). Early studies required prior density separation and extensive incubations to remove adherent cells (Linch, D.C, and Nathan, D.G. (1984), Nature 312 20/27: 775-777; Sieff, C.A., et al. (1985), Science 230: 1171-1173; Kannourakis, G. and Bol, S. (1987) Exp. Hematol 15:1103-1108.). More recent techniques are no less cumbersome; involving density separation steps followed by two partial lineage depletions (Winslow, J.M., et al. (1994), Bone Marrow Transplantation 14:265-271) or a partial lineage depletion using panning or FACS followed finally by positive selection using FACS (Carlo- Stella et al. 1994, Blood 84, 10 supple.:104a; Reading, C., et al. (1994), Blood 84, 10 supple.:399a). Most of these methods for lineage depletion lack effective antibody combinations against myeloid cells, erythrocytes and/or B-cells.

U.S. Patent Serial No. 5,087,570 describes a process for preparing a hematopoietic cell composition using a combination of positive and negative selection. The process relies on the use of antibody to the Sca-

1 antigen which is associated with murine clonogenic bone marrow precursors of thymocytes and progeny T-cells. The Sca-1 antibody is not useful in isolating human hematopoietic cells.

U.S. Patent Serial No. 5,137,809 describes a method and kit for identifying and analyzing lineages and maturational stages in normal hematopoietic cells. The method uses a first monoclonal antibody labeled with a fluorochrome to react with all leukocytes in a sample, and a second monoclonal antibody labeled with a second flourochrome to react with a subpopulation of leukocytes.

SUMMARY OF THE INVENTION

The present inventors have developed an antibody composition which is specifically adapted to enrich for hematopoietic stem cells and progenitor cells and remove tumor cells. The antibodies in the antibody composition are specific for selected markers associated with lineage committed or differentiated cells. Some of the markers are also expressed on some metastatic tumor cells. The composition contains antibodies specific for glycophorin A, CD3, CD24, CD16, CD14 and optionally CD45RA, CD38, CD36, CD2, CD19, CD20, CD22, CD29, CD56, CD66e, and/or CD66b, and antibodies specific for non-hematopoietic antigens expressed on tumor cells, preferably antibodies against antigens expressed on the surface of breast and lung carcinoma and neuroblastoma cells. The present inventors have shown that the purging antibody composition applied in one step to a sample of peripheral blood, bone marrow, or frozen bone marrow containing tumor cells, results in a greater than 50% recovery of human hematopoietic progenitor/stem cells with approximately a 3-5 log depletion of tumor cells.

The high level of enrichment obtained using the antibody composition of the invention, does not require additional enrichment or tumor purging steps, which would result in loss of, or damage to, progenitor and stem cells. The recovery of $CD34^+$ cells, $CD34^+CD38^-$ cells, colony forming cells, and LTCIC, is also much higher than with conventional multistep techniques.

The enrichment and recovery of human hematopoietic progenitor and stem cells using the antibody compositions of the invention in a negative selection technique has many advantages over conventional positive selection techniques. As mentioned above, highly enriched progenitor/stem cell preparations can be obtained using a single step. The human progenitor and stem cells obtained using the antibody composition of the invention are not labeled or coated with antibodies or modified making them highly suitable for transplantation and other therapeutic uses.

Broadly stated the present invention contemplates an antibody composition comprising antibodies specific for glycophorin A, CD3, CD24, CD16, CD14, and optionally one or more of CD45RA, CD36, CD38, CD56, CD2, CD19, CD20, CD22, CD29, CD66e and CD66b. Preferably the antibody composition contains antibodies specific for non-hematopoietic antigens expressed on tumor cells, most preferably antigens expressed on the surface of cells from breast and lung carcinoma, and neuroblastoma.

The present invention still further contemplates a process for enriching and recovering normal human hematopoietic progenitor cells and stem cells in a sample containing human hematopoietic differentiated, progenitor, and stem cells, and tumor cells comprising

(a) reacting the sample with an antibody composition containing antibodies capable of binding to the antigens glycophorin A, CD3, CD24, CD16, CD14, optionally one or more of CD45RA, CD38, CD36, CD56, CD2, CD19, CD20, CD22, CD29, CD66e, CD66b, and optionally antibodies specific for non-hematopoietic antigens expressed on tumor cells, under conditions so that cell conjugates are formed between the antibodies and the cells in the sample having the antigens expressed on the tumor cells;

(b) removing the cell conjugates; and

(c) recovering a cell preparation which is enriched in normal human hematopoietic progenitor cells and stem cells.

The present invention also relates to kits useful in performing processes of the invention comprising antibodies specific for

glycophorin A, CD3, CD24, CD16, CD14, optionally one or more of CD45RA, CD36, CD38, CD56, CD2, CD19, CD20, CD22, CD29, CD66e, CD66b, and optionally antibodies specific for non-hematopoietic antigens expressed on tumor cells and instructions for performing the processes of the invention.

The invention further relates to cell preparations obtained in accordance with the processes of the invention. The invention still further contemplates a method of using the antibody compositions of the invention in negative selection methods to recover a cell preparation which is enriched in human hematopoietic progenitor and stem cells.

The present invention also contemplates a tumor-enriching antibody composition which is adapted to enrich for tumor cells, in particular metastatic tumor cells. The composition is useful in the detection of non-hematopoietic tumor cells from blood and bone marrow of patients to aid in the detection of metastatic disease. The tumor-enriching antibody composition contains antibodies specific for selected markers associated with hematopoietic cells. In particular, the present inventors have found using a negative selection technique that an antibody composition containing antibodies specific for glycophorin A, CD3, CD19, CD36, CD56, CD14, CD16, CD66b, CD38, CD45, and optionally CD41, CD33, CD20, CD22, CD29, CD2, CD45RA, and/or CD10, gives a cell preparation highly enriched for non-hematopoietic tumor cells. The present inventors have shown that the tumor enriching antibody composition applied in one step to a sample of peripheral blood, frozen peripheral blood, or bone marrow containing tumor cells results in a greater than 3 log enrichment of the tumor cells.

The enrichment of non-hematopoietic tumor cells using the tumor-enriching antibody composition of the invention has many advantages. The composition provides for a greater than 3 log enrichment with good recovery of metastatic tumor cells. The recovered tumor cells are not labeled with antibody which can interfere with detection methods.

Therefore the invention contemplates a tumor-enriching antibody composition comprising antibodies specific for glycophorin A, CD3, CD19, CD36, CD56, CD14, CD16, CD66b, CD38, CD45, and optionally CD41, CD33, CD20, CD22, CD29, CD66e, CD2, CD45RA and/or CD10.

The present invention also contemplates a process for enriching for non-hematopoietic metastatic tumor cells in a sample containing the tumor cells and hematopoietic cells comprising

(a) reacting the sample with an antibody composition comprising antibodies specific for glycophorin A, CD3, CD19, CD36, CD56, CD14, CD16, CD66b, CD38, CD45, and optionally CD41, CD33, CD20, CD22, CD29, CD2, CD45RA and/or CD10, under conditions so that conjugates are formed between the antibodies and hematopoietic cells in the sample expressing the antigens glycophorin A, CD3, CD19, CD36, CD56, CD14, CD16, CD66b, CD38, CD45, and optionally CD41, CD33, CD20, CD22, CD29, CD2, CD45RA, and/or CD10;

(b) removing the cell conjugates, and

(c) recovering a cell preparation enriched in the tumor cells.

The invention still further contemplates a process for detecting metastatic tumor cells in a sample containing the metastatic tumor cells and hematopoietic cells comprising

(a) reacting the sample with an antibody composition comprising antibodies specific for glycophorin A, CD3, CD19, CD36, CD56, CD14, CD16, CD66b, CD38, CD45, and optionally CD41, CD33, CD20, CD22, CD29, CD2, CD45RA, and/or CD10, under conditions so that conjugates are formed between the antibodies and hematopoietic cells in the sample expressing the antigens glycophorin A, CD3, CD19, CD36, CD56, CD14, CD16, CD66b, CD38, CD45, and optionally CD41, CD33, CD20, CD22, CD29, CD2, CD45RA, and/or CD10,

(b) removing the cell conjugates, and

(c) recovering a cell preparation enriched in the tumor cells.

The present invention also relates to kits useful in performing processes of the invention comprising antibodies specific for glycophorin A, CD3, CD19, CD36, CD56, CD14, CD16, CD66b, CD38, CD45, and optionally CD41, CD33, CD20, CD22, CD29, CD2, CD45RA, and/or CD10, and instructions for performing the tumor cell enriching processes of the invention.

The invention further relates to metastatic cell preparations obtained in accordance with the processes of the invention. The invention still further contemplates a method of using the tumor-enriching antibody compositions of the invention in negative selection methods to recover a cell preparation which is enriched in non-hematopoietic metastatic tumor cells.

These and other aspects of the present invention will become evident upon reference to the following detailed description and attached drawings. In addition, reference is made herein to various publications, which are hereby incorporated by reference in their entirety.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will now be described with reference to the accompanying drawings, in which:

Figure 1 is a schematic representation of magnetic cell labelling using tetrameric antibody complexes and colloidal dextran iron.

DETAILED DESCRIPTION OF THE INVENTION

I. HEMATOPOIETIC CELL TYPES AND TUMOR CELLS

The term "differentiated cells" used herein refers to human hematopoietic cells which have limited or no proliferative capacity. Differentiated cells represent specialized end cells that are found in blood, and their immediate precursors.

The term "progenitor cells" used herein refers to cells which are the immediate precursors of the differentiating cells. Most of the progenitor cells differentiate along a single lineage but they may have quite extensive proliferative capacity. Progenitor cells appear morphologically as

blast cells, and they typically do not have specific features of the hematopoietic lineage to which they are committed.

The term "stem cells" used herein refers to the cells from which progenitor cells are derived. Stem cells are defined by their ability to self-renew as well as to generate daughter cells of any of the hematopoietic lineages. Stem cells with long term hematopoietic reconstituting ability can be distinguished by a number of physical and biological properties from differentiated cells and progenitor cells (Hodgson, G.S. & Bradley, T.R., Nature, Vol. 281, pp. 381-382; Visser et al., J. Exp. Med., Vol. 59, pp. 1576-1590, 1984; Spangrude et al., Science, Vol. 241, pp. 58-62, 1988; Szilvassy et al., Blood, Vol. 74, pp. 930-939, 1989; Ploemacher, R.E. & Brons, R.H.C., Exp. Hematol., Vol. 17, pp. 263-266, 1989).

The presence of stem cells and progenitor cells in a cell preparation may be detected by their ability to produce colony-forming cells in culture. They may also be detected by screening for the CD34 antigen which is a positive marker for early hematopoietic cells including colony forming cells and stem cells. Primitive hematopoietic stem cells with long term hematopoietic reconstituting ability can be identified by determining the number of clonogenic cells present after 5 to 8 weeks in long term cultures (Sutherland et al., Blood, Vol. 74, p. 1563, 1986; Udomsakdi et al., Exp. Hematol., Vol. 19, p. 338, 1991; and, Sutherland et al., Proc. Natl. Acad. Sci., Vol. 87, p. 3584, 1990).

Tumor cells which may be removed from a sample using the antibody compositions and processes described herein include tumor cells which have non-hematopoietic antigens or markers expressed on their surfaces i.e. antigens that distinguish the tumor cells from hematopoietic progenitor cells and stem cells. For example, specific markers have been found to be expressed on tumor cells such as breast and lung carcinoma, and neuroblastoma. Table 1 lists specific examples of antibodies which recognize non-hematopoietic antigens expressed on tumor cells.

Some metastatic tumor cells express hematopoietic lineage markers or antigens, for example, tumor cells from B-lymphomas, multiple

myeloma, some chronic lymphocytic leukemias (CLL), and some acute lymphocytic leukemias (ALL) express B-cell markers such as CD22, CD20, CD29, and T cells from ALL and CLL express T-cell markers, and antibodies to these antigens may be included in the antibody compositions of the invention to remove tumor cells expressing the hematopoietic lineage antigens.

II. ANTIBODY COMPOSITIONS

As hereinbefore mentioned, the present invention relates to an antibody composition comprising antibodies specific for glycophorin A, CD3, CD24, CD16, CD14, and optionally specific for CD45RA, CD36, CD38, CD56, CD2, CD19, CD20, CD22, CD29, CD66e, CD66b, and/or non-hematopoietic antigens expressed on tumor cells.

The antibodies in the antibody composition are selected based on the desired degree of enrichment and the types of cells which are prevalent in a particular sample. For example, in blood samples T cells are preferably removed but it may be desirable to leave some NK cells. The T cell load in a bone marrow sample will be much lower and including anti-T cell antibodies in the composition may not be absolutely necessary for obtaining an acceptable degree of debulking. Generally an antibody composition having at least two antibodies against T cells and two antibodies against B cells provides maximum enrichment.

In an embodiment of the invention the antibody composition contains antibodies specific for glycophorin A, CD3, CD24, CD16, CD14, CD56, CD2, CD19, and CD66b. Preferably, the compositions contain antibodies specific for non-hematopoietic antigens, most preferably antigens expressed on breast and lung carcinoma and neuroblastoma cells.

Pluripotent stem cells and committed progenitors express CD34, and this CD34 compartment can be subdivided using antibodies to a variety of cell surface markers. Stem cells co-purify in a population of CD34$^+$ cells which lack or have low expression of certain lineage markers (CD38, CD33, CD45RA, CD71, CD36 and HLA-DR) (Craig et al. 1994, British Journal of Haematology, 88:24-30; Lansdorp, P.Al. and Dragowska, W. (1992)

J. Exp. Med. 175:1501-1509; Sutherland, H.J., et al. (1989) Blood 74.1563-1570). Antibodies recognizing these antigens can be included in the antibody composition to further enrich for stem cells, while losing some of the committed mature $CD34^{+}$ cells. Preferably, anti-CD45RA, anti-CD38 and anti-CD36 are included in the antibody composition.

In an embodiment of the invention the antibody composition contains antibodies specific for glycophorin A, CD3, CD24, CD16, CD14, CD56, CD2, CD19, CD66b, CD36, CD45RA, and CD38, and optionally antibodies specific for non-hematopoietic antigens, most preferably antigens expressed on breast and lung carcinoma and neuroblastoma cells.

The invention also contemplates a tumor-enriching antibody composition comprising antibodies specific for glycophorin A, CD3, CD19, CD36, CD56, CD14, CD16, CD66b, CD38, CD45, and optionally CD41, CD33, CD20, CD22, CD29, CD66e, CD2, CD45RA, and/or CD10. In an embodiment of the invention the tumor-enriching antibody composition comprises glycophorin A, CD3, CD2, CD19, CD36, CD56, CD14, CD16, CD66b, CD38, CD45RA, and CD45.

Within the context of the present invention, antibodies are understood to include monoclonal antibodies and polyclonal antibodies, antibody fragments (e.g., Fab, and $F(ab')_2$) and chimeric antibodies. Antibodies are understood to be reactive against a selected antigen on the surface of a hematopoietic cell, differentiated cell or tumor cell if they bind with an appropriate affinity (association constant), e.g. greater than or equal to 10^7 M^{-1}.

Polyclonal antibodies against selected antigens on the surface of hematopoietic cells, differentiated cells or tumor cells may be readily generated by one of ordinary skill in the art from a variety of warm-blooded animals such as horses, cows, various fowl, rabbits, mice, hamsters, or rats. For example, a mammal, (e.g., a mouse, hamster, or rabbit) can be immunized with an immunogenic form of an antigen which elicits an antibody response in the mammal. Techniques for conferring

immunogenicity on an antigen include conjugation to carriers or other techniques well known in the art. For example, the antigen can be administered in the presence of adjuvant. The progress of immunization can be monitored by detection of antibody titers in plasma or serum. Following immunization, antisera can be obtained and polyclonal antibodies isolated from the sera.

Monoclonal antibodies are preferably used in the antibody compositions of the invention. Monoclonal antibodies specific for selected antigens on the surface of hematopoietic cells, differentiated cells or tumor cells may be readily generated using conventional techniques. For example, monoclonal antibodies may be produced by the hybridoma technique originally developed by Kohler and Milstein (Nature 256, 495-497 (1975)). (See also U.S. Patent Nos. RE 32,011, 4,902,614, 4,543,439, and 4,411,993 which are incorporated herein by reference; see also Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses, Plenum Press, Kennett, McKearn, and Bechtol (eds.), 1980, and Antibodies: A Laboratory Manual, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988).

Other techniques may also be utilized to construct monoclonal antibodies (for example, see William D. Huse et al., "Generation of a Large Combinational Library of the Immunoglobulin Repertoire in Phage Lambda," Science 246:1275-1281, December 1989; see also L. Sastry et al., "Cloning of the Immunological Repertoire in Escherichia coli for Generation of Monoclonal Catalytic Antibodies: Construction of a Heavy Chain Variable Region-Specific cDNA Library," Proc Natl. Acad. Sci USA 86:5728-5732, August 1989; Kozbor et al., Immunol. Today 4, 72 (1983) re the human B-cell hybridoma technique; Cole et al. Monoclonal Antibodies in Cancer Therapy (1985) Allen R. Bliss, Inc., pages 77-96 re the EBV-hybridoma technique to produce human monoclonal antibodies; and see also Michelle Alting-Mees et al., "Monoclonal Antibody Expression Libraries: A Rapid Alternative to Hybridomas," Strategies in Molecular Biology 3:1-9, January 1990). Hybridoma cells can be screened

immunochemically for production of antibodies specifically reactive with an antigen, and monoclonal antibodies can be isolated.

The term "antibody" as used herein is intended to include antibody fragments which are specifically reactive with specific antigens on the surface of hematopoietic cells, differentiated cells or tumor cells. Antibodies can be fragmented using conventional techniques and the fragments screened for utility in the same manner as described above for whole antibodies. For example, $F(ab')_2$ fragments can be generated by treating antibody with pepsin. The resulting $F(ab')_2$ fragment can be treated to reduce disulfide bridges to produce Fab' fragments.

The invention also contemplates chimeric antibody derivatives, i.e., antibody molecules that combine a non-human animal variable region and a human constant region. Chimeric antibody molecules can include, for example, the antigen binding domain from an antibody of a mouse, rat, or other species, with human constant regions. A variety of approaches for making chimeric antibodies have been described and can be used to make chimeric antibodies containing the immunoglobulin variable region which recognizes selected antigens on the surface of differentiated cells or tumor cells. See, for example, Morrison et al., Proc. Natl. Acad. Sci. U.S.A. 81,6851 (1985); Takeda et al., Nature 314, 452 (1985), Cabilly et al., U.S. Patent No. 4,816,567; Boss et al., U.S. Patent No. 4,816,397; Tanaguchi et al., European Patent Publication EP171496; European Patent Publication 0173494, United Kingdom patent GB 2177096B.

Binding partners may be constructed utilizing recombinant DNA techniques. Within one embodiment, the genes which encode the variable region from a hybridoma producing a monoclonal antibody of interest are amplified using nucleotide primers for the variable region. These primers may be synthesized by one of ordinary skill in the art, or may be purchased from commercially available sources. The primers may be utilized to amplify heavy or light chain variable regions, which may then be inserted into vectors such as ImmunoZAP™ H or ImmunoZAP™ L (Stratacyte), respectively. These vectors may then be introduced into *E. coli*

for expression. Utilizing these techniques, large amounts of a single-chain protein containing a fusion of the V_H and V_L domains may be produced (See Bird et al., Science 242:423-426, 1988). In addition, such techniques may be utilized to change a "murine" antibody to a "human" antibody, without altering the binding specificity of the antibody.

Antibodies against selected antigens on the surface of hematopoietic cells, differentiated cells or tumor cells may also be obtained from commercial sources.

Antibodies may be selected for use in the antibody compositions of the invention based on their ability to deplete targeted hematopoietic cells, differentiated cells and/or tumor cells and recover non-targeted cells (i.e. normal progenitor and stem cells, specific differentiated cells, or tumor cells), in magnetic cell separations as more particularly described herein, and in co-pending U.S. Patent Application Serial No. 08/185,064, which is incorporated in its entirety herein by reference. In general, an antibody is selected that gives greater than 3 log depletion of hematopoietic cells, differentiated cells or tumor cells. In an embodiment of the invention for enriching for hematopoietic stem cells and progenitor cells, an antibody is selected that gives greater than 3 log depletion of differentiated cells or tumor cells, with greater than 75% recovery of $CD34^+$ cells (bone marrow, mobilized blood and cord blood) or non-targeted lymphocytes (steady state blood), in test magnetic cell separations as described herein.

The anti-glycophorin A antibodies contained in the antibody compositions of the invention are used to label erythrocytes. Examples of monoclonal antibodies specific for glycophorin A are 10F7MN (U.S. Patent No. 4,752,582, Cell lines: ATCC accession numbers HB-8162), and D2.10 (Immunotech, Marseille, France). The concentration of antiglycophorin A antibodies used in the antibody compositions are generally less than the concentration that will cause agglutination (i.e. 3-10μg/ml). Preferably the concentration of antiglycophorin A antibodies

used in the antibody compositions is between about 0.5 to 5 μg/ml, preferably 1 to 2 μg/ml.

Monoclonal antibodies against CD24, CD3, CD19, CD20, CD22, CD29, CD56, CD2 in the antibody compositions of the invention are used to label B and T lymphocytes and NK cells. Examples of monoclonal antibodies specific for CD24, CD3, CD19, CD20, CD22, CD56, and CD2, are 32D12 (Dr. Steinar Funderud, Institute for Cancer Research, Dept. of Immunology, Oslo, Norway,) and ALB9 (Immunotech, Marseille, France); UCHT1 (Immunotech, Marseille, France) and Leu-4 (Becton Dickinson, Mountain View, Calif.); J4.119 (Immunotech, Marseille, France) and Leu-12 (Becton Dickinson, Mountain View, Calif.); MEM97 (Dr. Horejsi, Institute of Molecular Genetics Academy of Sciences of the Czech Republic, Praha, Czech Republic, or Cedarlane Laboratories, Hornby, Ontario, Canada) and Leu-16 (Becton Dickinson, Mountain View, Calif.); SJ10.1H11 (Immunotech, Marseille, France); T199 (Immunotech, Marseille, France); and 6F10.3 (Immunotech, Marseille, France), respectively. The concentration of each of the monoclonal antibodies against CD24, CD3, CD19, CD20, CD56, CD2 contained in the antibody composition is between about 0.5 to 5 μg/ml, preferably 2 to 3 μg/ml.

Monoclonal antibodies against CD14, CD16, CD66e and CD66b in the antibody compositions of the invention are used to label monocytes and granulocytes. Examples of monoclonal antibodies specific for CD14, CD16, CD66e and CD66b, are MEM15 and MEM18 (Dr. Vaclav Horejsi, Institute of Molecular Genetics Academy of Sciences of the Czech Republic, Praha, Czech Republic; Cedarlane Laboratories, Hornby, Ontario, Canada); MEM154 (Dr. Vaclav Horejsi, Institute of Molecular Genetics Academy of Sciences of the Czech Republic, Praha, Czech Republic; Cedarlane Laboratories, Hornby, Ontario, Canada), Leu-11a (Becton Dickinson, Mountain View, Calif.), and 3G8 (Immunotech, Marseille, France); CLB/gran10 (CLB, Central Laboratory of the Netherlands, Red Cross Blood Transfusion Service); and, B13.9 (CLB, Central Laboratory of the Netherlands, Red Cross Blood Transfusion Service) and 80H3

(Immunotech, Marseille, France), respectively. The concentration of each of the monoclonal antibodies against CD14, CD16, CD66e and CD66b contained in the antibody compositions is between about 0.5 to 5 μg/ml, preferably 2-3 μg/ml.

Monoclonal antibodies against CD45RA, CD38 and CD36 are used to label T-cells, B-cells plasma cells, granulocytes, platelets, monocytes, differentiated erythroid precursors, and some committed mature progenitors, to further enrich for stem cells. Examples of monoclonal antibodies against CD45RA, CD38 and CD36 are 8D2.2 (StemCell Technologies, Vancouver, Canada, Craig et al., 1994, British Journal of Haematology, 88:24-30.), Leu-18 (Becton Dickinson, Mountain View, Calif.); T16 (Immunotech, Marseille, France); and, FA6.152 (Immunotech, Marseille, France) and IVC7 (CLB, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service), respectively. The concentration of each of the monoclonal antibodies against CD45RA, CD38, and CD36 contained in the antibody compositions is between about 0.5 to 5 μg/ml, preferably 1 to 3 μg/ml.

Monoclonal antibodies against CD45 are used to label leukocytes eg. lymphocytes, monocytes, granulocytes - essentially all nucleated cells in blood and bone marrow. CD45 is not on mature erythrocytes and plasma cells. An example of an anti-CD45 antibody is J33 (Immunotech, Marseille, France). The concentration of monoclonal antibodies against CD45 in the antibody composition of the invention is between about 0.5 to 5.0 μg/ml, preferably 1 to 3 μg/ml.

Monoclonal antibodies against CD41 are used to label megakaryocytes and platelets. An example of an anti-CD41 antibody is SZ22 (Immunotech, Marseille, France). The concentration of monoclonal antibodies against CD41 in the antibody composition of the invention is between about 0.5 to 5.0 μg/ml, preferably 1 to 3 μg/ml.

CD33 is a myeloid marker found on monocytes, granulocytes, and macrophage precursors. Therefore, monoclonal

antibodies against CD33 are used to label monocytes, granulocytes, and macrophage precursors. An example of an anti-CD33 antibody is D3HL60.251 (Immunotech, Marseille, France). The concentration of monoclonal antibodies against CD33 in the antibody composition of the invention is between about 0.5 to 5.0 μg/ml, preferably 1 to 3 μg/ml.

Table 2 sets out the most preferred monoclonal antibodies specific for differentiated cells, their sources and concentrations, for use in the antibody compositions of the invention. Table 1 sets out the most preferred monoclonal antibodies specific for tumor cells, and commercial sources/references for the antibodies.

A preferred antibody composition for removing differentiated hematopoietic cells and breast and lung carcinoma cells from a sample comprises the monoclonal antibodies D2.10, UCHT1, MEM15, 3G8, ALB9, 80H3, J4.119, 6F10.3, T199, 8D2.2, T16 and FA6.152 or the monoclonal antibodies 10F7MN, UCHT1, 32D12, MEM154, MEM15, 80H3 or B13.29, T199, 6F10.3, J4.119, and optionally, 8D2.2, T16 and IVC7, and one or more of the monoclonal antibodies specific for an antigen on the surface of a breast or lung carcinoma as set forth in Table 1. Most preferably the monoclonal antibodies specific for an antigen on the surface of cells from a breast carcinoma used in a composition of the invention are one or more of 5E11, H23A, 6E7, RAR, and BRST1.

A preferred antibody composition for removing differentiated hematopoietic cells and neuroblastoma cells from a sample comprises the monoclonal antibodies D2.10, UCHT1, MEM15, 3G8, ALB9, 80H3, J4.119, 6F10.3, T199, and optionally 8D2.2, T16 and FA6.152 or the monoclonal antibodies 10F7MN, UCHT1, 32D12, MEM154, MEM15, 80H3 or B13.29, T199, 6F10.3, J4.119, and optionally, 8D2.2, T16 and IVC7, and one or more of the monoclonal antibodies specific for an antigen on the surface of cells from a neuroblastoma as set forth in Table 1.

Preferred antibody compositions for enriching for non-hematopoietic metastatic tumor cells from a sample containing

hematopoietic cells and non-hematopoietic metastatic tumor cells comprise the monoclonal antibodies D2.10, UCHT1, MEM15, 3G8, 80H3, J4119, 6F10.3, T199, 8D2.2, T16, FA6.152, and J33; or the monoclonal antibodies 10F7MN, UCHT1, MEM15, MEM154, B13.29, J4119, 6F10.3, T199, 8D2.2 T16, IVC7, and J33.

III. PROCESS FOR PREPARING CELL PREPARATIONS ENRICHED IN PROGENITOR/STEM CELLS, OR TUMOR CELLS

The antibody compositions of the invention may be used to enrich and recover cell preparations enriched in human hematopoietic stem cells and progenitor cells. In accordance with a process of the invention, a sample is reacted with an antibody composition of the invention; under suitable conditions, cell conjugates form between the antibodies contained in the antibody composition which are specific for selected antigens on the surface of differentiated cells and/or tumor cells, and the cells in the sample containing the antigens on their surface; and the cell conjugates are removed.

The antibody composition of the invention which is a tumor-enriching antibody composition may be used to enrich for non-hematopoietic metastatic tumor cells in a sample. In accordance with a process of the invention a sample containing non-hematopoietic metastatic tumor cells and hematopoietic cells is reacted with a tumor-enriching antibody composition; under suitable conditions conjugates are formed between the antibodies in the composition which are specific for antigens on the surface of hematopoietic cells in the sample expressing the antigens; and the cell conjugates are removed to provide a cell preparation enriched in the tumor cells.

Conditions which permit the formation of cell conjugates may be selected having regard to factors such as the nature and amounts of the antibodies in the antibody composition, and the estimated concentration of targeted differentiated cells in the sample.

The antibodies in the antibody compositions may be labelled with a marker or they may be conjugated to a matrix. Examples of

markers are biotin, which can be removed by avidin bound to a support, and fluorochromes, e.g. fluorescein, which provide for separation using fluorescence activated sorters. Examples of matrices are magnetic beads, which allow for direct magnetic separation (Kernshead 1992), panning surfaces e.g. plates, (Lebkowski, J.S, et al., (1994), J. of Cellular Biochemistry supple. 18b:58), dense particles for density centrifugation (Van Vlasselaer, P., Density Adjusted Cell Sorting (DACS), A Novel Method to Remove Tumor Cells From Peripheral Blood and Bone Marrow StemCell Transplants. (1995) 3rd International Symposium on Recent Advances in Hematopoietic Stem Cell Transplantation-Clinical Progress, New Technologies and Gene Therapy, San Diego, CA), adsorption columns (Berenson et al. 1986, Journal of Immunological Methods 91:11-19.), and adsorption membranes (Norton et al. 1994). The antibodies may also be joined to a cytotoxic agent such as complement or a cytotoxin, to lyse or kill the targeted cells.

The antibodies in the antibody compositions may be directly or indirectly coupled to a matrix. For example, the antibodies in the compositions of the invention may be chemically bound to the surface of magnetic particles for example, using cyanogen bromide. When the magnetic particles are reacted with a sample, conjugates will form between the magnetic particles with bound antibodies specific for antigens on the surfaces of the hematopoietic cells, differentiated cells and/or tumor cells, and the hematopoietic cells, differentiated cells and/or tumor cells having the antigens on their surfaces.

Alternatively, the antibodies may be indirectly conjugated to a matrix using antibodies. For example, a matrix may be coated with a second antibody having specificity for the antibodies in the antibody composition. By way of example, if the antibodies in the antibody composition are mouse IgG antibodies, the second antibody may be rabbit anti-mouse IgG.

The antibodies in the antibody compositions may also be incorporated in antibody reagents which indirectly conjugate to a matrix. Examples of antibody reagents are bispecific antibodies, tetrameric antibody complexes, and biotinylated antibodies.

Bispecific antibodies contain a variable region of an antibody in an antibody composition of the invention, and a variable region specific for at least one antigen on the surface of a matrix. The bispecific antibodies may be prepared by forming hybrid hybridomas. The hybrid hybridomas may be prepared using the procedures known in the art such as those disclosed in Staerz & Bevan, (1986, PNAS (USA) 83: 1453) and Staerz & Bevan, (1986, Immunology Today, 7:241). Bispecific antibodies may also be constructed by chemical means using procedures such as those described by Staerz et al., (1985, Nature, 314:628) and Perez et al., (1985 Nature 316:354), or by expression of recombinant immunoglobulin gene constructs.

A tetrameric immunological complex may be prepared by mixing a first monoclonal antibody which is capable of binding to at least one antigen on the surface of a matrix, and a second monoclonal antibody from the antibody composition of the invention. The first and second monoclonal antibody are from a first animal species. The first and second antibody are reacted with an about equimolar amount of monoclonal antibodies of a second animal species which are directed against the Fc-fragments of the antibodies of the first animal species. The first and second antibody may also be reacted with an about equimolar amount of the $F(ab')_2$ fragments of monoclonal antibodies of a second animal species which are directed against the Fc-fragments of the antibodies of the first animal species. (See U.S. Patent No. 4,868,109 to Lansdorp, which is incorporated herein by reference for a description of tetrameric antibody complexes and methods for preparing same).

The antibodies of the invention may be biotinylated and indirectly conjugated to a matrix which is labelled with (strept) avidin. For example, biotinylated antibodies contained in the antibody compositions of the invention may be used in combination with magnetic iron-dextran

particles that are covalently labelled with (strept) avidin (Miltenyi, S. et al., Cytometry 11:231, 1990). Many alternative indirect ways to specifically cross-link the antibodies in the antibody compositions and matrices would also be apparent to those skilled in the art.

In an embodiment of the invention, the cell conjugates are removed by magnetic separation using magnetic particles. Suitable magnetic particles include particles in ferrofluids and other colloidal magnetic solutions. "Ferrofluid" refers to a colloidal solution containing particles consisting of a magnetic core, such as magnetite (Fe_3O_4) coated or embedded in material that prevents the crystals from interacting. Examples of such materials include proteins, such as ferritin, polysaccharides, such as dextrans, or synthetic polymers such as sulfonated polystyrene cross-linked with divinylbenzene. The core portion is generally too small to hold a permanent magnetic field. The ferrofluids become magnetized when placed in a magnetic field. Examples of ferrofluids and methods for preparing them are described by Kemshead J.T. (1992) in J. Hematotherapy, 1:35-44, at pages 36 to 39, and Ziolo et al. Science (1994) 257:219 which are incorporated herein by reference. Colloidal particles of dextran-iron complex are preferably used in the process of the invention. (See Molday, R.S. and McKenzie, L.L. FEBS Lett. 170:232, 1984; Miltenyi et al., Cytometry 11:231, 1990; and Molday, R.S. and MacKenzie, D., J. Immunol. Methods 52:353, 1982; Thomas et al., J. Hematother. 2:297 (1993); and U.S. Patent No. 4,452,733, which are each incorporated herein by reference).

Figure 1 is a schematic representation of magnetic cell labeling using tetrameric antibody complexes and colloidal dextran iron.

In accordance with a magnetic separation method, the sample containing the progenitor and stem cells to be recovered, is reacted with the above described antibody reagents, preferably tetrameric antibody complexes, so that the antibody reagents bind to the targeted differentiated cells, tumor cells, and/or hematopoietic cells present in the sample to form cell conjugates of the targeted differentiated cells, tumor cells, and/or hematopoietic cells and the antibody reagents. The reaction conditions are

selected to provide the desired level of binding of the targeted differentiated cells, tumor cells, and/or hematopoietic cells and the antibody reagents. Preferably the sample is incubated with the antibody reagents for a period of 5 to 60 minutes at either 4° or ambient room temperature. The concentration of the antibody reagents is selected depending on the estimated concentration of the targeted differentiated cells in the sample. Generally, the concentration is between about 0.1 to 50 μg/ml of sample. The magnetic particles are then added and the mixture is incubated for a period of about 5 minutes to 30 minutes at the selected temperature. The sample is then ready to be separated over a magnetic filter device. Preferably, the magnetic separation procedure is carried out using the magnetic filter and methods described in co-pending U.S. patent application serial No. 08/185,064 to Lansdorp and Thomas which is incorporated in its entirety herein by reference.

The sample containing the magnetically labelled cell conjugates is passed through the magnetic filter in the presence of a magnetic field. In a preferred embodiment of the invention, the magnet is a solenoid electromagnet with a 3" diameter bore and having a magnetic field of 0.5-2 Tesla. The magnetically labelled cell conjugates are retained in the high gradient magnetic column and the materials which are not magnetically labelled flow through the column after washing with a buffer.

A preparation containing non-magnetically labelled cells may be analyzed using procedures such as flow cytometry. The ability of a cell preparation containing progenitor and stem cells to produce colony-forming cells or long term culture initiating cells (LTCIC) in culture may also be assessed. The efficiency of the separation procedure may also be determined by monitoring the recovery of $CD34^+$ cells, $CD34^+$ $CD38^-$ cells and colony forming cells.

The above described magnetic separation methods may be adapted for enriching for tumor cells in a sample containing tumor cells and hematopoietic cells.

IV. Uses of the Compositions and Processes of the Invention

The device and processes of the invention may be used in the processing of biological samples including blood in particular, cord blood and whole blood. It has also been found that the antibody compositions of the invention can be used to prepare hematopoietic progenitor and stem cell preparations and tumor cell preparations from bone marrow samples, including previously frozen bone marrow samples.

The processes of the invention may be used to deplete or purge erythrocytes, B and T lymphocytes, monocytes, NK cells, granulocytes, and tumor cells from samples to prepare hematopoietic progenitor and stem cell preparations for use in transplantation as well as other therapeutic methods that are readily apparent to those of skill in the art. For example, bone marrow or blood can be harvested from a donor in the case of an allogenic transplant and enriched for progenitor and stem cells by the processes described herein.

Using the process of the invention it is possible to recover a highly purified preparation of human hematopoietic stem/progenitor cells. In particular, a hematopoietic cell population containing greater than 50% of the hematopoietic progenitor/stem cells present in the original sample, and which is depleted of differentiated cells and tumor cells in the original sample by greater than 3 logarithms may be obtained. The human hematopoietic progenitor and stem cells in the preparation are not coated with antibodies, or modified making them highly suitable for transplantation and other therapeutic uses that are readily apparent to those skilled in the art.

The cell preparations obtained using the processes of the invention may be used to isolate and evaluate factors associated with the differentiation and maturation of human hematopoietic cells. The cell preparations may also be used to determine the effect of a substance on cell growth and/or differentiation into a particular lineage.

The tumor-enriching antibody composition of the invention is adapted to enrich for tumor cells, in particular non-hematopoietic metastatic tumor cells. The composition is useful in the

detection of non-hematopoietic tumor cells from blood, bone marrow, and peritoneal and pleural effusions, of patients to aid in the diagnosis and detection of metastatic disease, monitoring the progression of metastatic disease, or monitoring the efficacy of a treatment. The tumor enriching antibody composition applied in one step to a sample of peripheral blood, frozen peripheral blood, or bone marrow containing tumor cells results in a greater than 3 log enrichment of the tumor cells.

The following non-limiting examples are illustrative of the present invention:

EXAMPLES

EXAMPLE 1

PURGING BREAST CARCINOMA CELLS (BT20 or T47D CELLS)

Tetramers of anti-breast carcinoma antibodies as shown in Table 1 were combined with a progenitor enrichment cocktail (D2.10, UCHT1, MEM15, 3G8, ALB9, 80H3, J4.119, 6F10.3, T199, and optionally 8D2.2, T16 and FA6.152, or 10F7MN, UCHT1, 32D12, MEM154, MEM15, 80H3 or B13.9, T199, 6F10.3, J4.119, and optionally, 8D2.2, T16 and IVC7) to produce a cocktail for breast carcinoma purging and debulking. Including the lineage depletion increases the degree of tumor purge over that seen with just anti-tumor antibodies alone (Table 3). Breast carcinoma cell lines were added to previously frozen marrow, peripheral blood leukapheresis or fresh bone marrow. Tumor cell purges were performed using the anti-breast carcinoma antibodies indicated in Table 3 with and without the standard lineage depletion (progenitor enrichment cocktail). The recovery of hematopoietic progenitors during lineage depletion is given in Table 4. Enrichment of progenitors was generally 50 to 100 fold.

In summary, purging tumor cells for hematopoietic progenitors in a one step selection using the antibody cocktail as indicated in Table 3 achieves a much higher degree of tumor cell purging than positive selection techniques while offering a similar degree of progenitor enrichment. The recoveries of hematopoietic progenitor cells in a lineage depletion are greater than those typically seen with positive selection.

EXAMPLE 2

Cells from the CAMA breast carcinoma cell line were mixed with previously frozen bone marrow (BM) or peripheral blood (PB) and processed with the enrichment antibody composition (D2.10, UCHT1, MEM15, 3G8, 8OH3, J4119, 6F10.3, T199, 8D2.2, T16, FA6.152, and J33) in a one step magnetic depletion. The frequency of CAMA cells in the start suspension varied from 2% to $2/10^6$ cells (Table 5). CAMA cells were enriched 2-3 log.

While what is shown and described herein constitutes various preferred embodiments of the subject invention, it will be understood that various changes can be made to such embodiments without departing from the subject invention, the scope of which is defined in the appended claims.

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Table 1 Antibodies Recognizing Non-Hematopoietic Antigens Expressed on Tumor Cells.

Disease	Antibody	Antigen	Supplier/Developer
Breast and Lung Carcinoma	5E11	unknown, breast carcinoma	STI
	6E7	unknown, breast carcinoma	STI
	H23A	unknown, breast carcinoma	ATCC
	RAR9941	epithelial glycoprotein	Baxter, Germany
	RAR9948	epithelial glycoprotein	Baxter, Germany
	RAR9938	crb2	Baxter, Germany
	C13B5	crb2	Immunotech, Marseille, France
	BRST 1	BCA 225	ID Labs
	BRST 3	TAG-72	ID Labs
	CA15.3	MAM-6, mucin	ID Labs
	CA27.29	MAM-6, mucin	Cedarlane
Neuroblastoma	UJ13A	unknown	Hurko and Walsh (1983 Neurology 33:734
	UJ181.4	unknown	-
	UJ223.8	unknown	-
	UJ127.11	unknown	-
	5.1.H11	unknown	-
	390,459	unknown	R.C. Seeger, L.A. Children's Hospital, Calif.
	BA-1.2	unknown	-
	HSAN 1.2	unknown	Reynolds and Smith (1982 Hybridomas in Cancer p235

Table 2: Antibodies used in Lineage Depletions

Antigen	Antibody	Source	Concentration ug/ml
glycophorin	10F7MN* D2.10	U.S. Patent No. 4,752,582 IMMUNOTECH, Marseille, France	1 2
CD2	6F10.3	IMMUNOTECH, Marseille, France	3
CD3	UCHT1 Leu-4	IMMUNOTECH, Marseille, France Becton Dickinson Immunocytometry, Mountain View, Calif.	3
CD4	Leu-3a	Becton Dickinson Immunocytometry, Mountain View, Calif.	
CD8	Leu-2a OKT3	Becton Dickinson Immunocytometry, Mountain View, Calif. BioDesigns	
CD14	MEM 15 MEM 18	Dr. Vaclav Horejsi, Institute of Molecular Genetics Academy of Sciences of the Czech Republic, Praha, Czech Republic; Cedarlane Laboratories Hornby, Ontario, Canada	2 2
CD16	MEM 154* 3G8 Leu-11a	Dr. Vaclav Horejsi, Institute of Molecular Genetics Academy of Sciences of the Czech Republic, Praha, Czech Republic; Cedarlane Laboratories Hornby, Ontario, Canada IMMUNOTECH, Marseille, France Becton Dickinson Immunocytometry, Mountain View, Calif.	2 3

CD19	J4.119 Leu-12	IMMUNOTECH, Marseille, France Becton Dickinson Immunocytometry, Mountain View, Calif.	3
CD20	MEM97 Leu-16	Dr. Vaclav Horejsi, Institute of Molecular Genetics Academy of Sciences of the Czech Republic, Praha, Czech Republic; Cedarlane Laboratories Hornby, Ontario, Canada Becton Dickinson Immunocytometry, Mountain View, Calif.	3
CD24	32D12* ALB9	Dr. Steinar Funderud, Institute for Cancer Research, Dept. of Immunology, Oslo, Norway IMMUNOTECH, Marseille, France	2 3
CD33	D3HL60.251	Immunotech	3
CD36	FA6.152 IVC7	IMMUNOTECH, Marseille, France CLB, Central Laboratory of the Netherlands, Red Cross Blood Transfusion Service	3
CD38	T16	IMMUNOTECH, Marseille, France	3
CD41	PI1.64 S222	Kaplan, 5th International Workshop on Human Leukocyte Differentiation Antigens Immunotech	3 3
CD42a	Bebl	Becton Dickinson Immunocytometry, Mountain View, Calif.	3
CD45	J33	Immunotech	3

CD45RA	8D2.2 Leu-18	Craig et al. 1994, StemCell Technologies, Vancouver, Canada Becton Dickinson Immunocytometry, Mountain View, Calif.	1
CD56	T199	IMMUNOTECH, Marseille, France	3
CD66e	CLB/gran10	CLB, Central Laboratory of the Netherlands, Red Cross Blood Transfusion Service	3
CD66b	B13.29 8CH3	CLB, Central Laboratory of the Netherlands, Red Cross Blood Transfusion Service IMMUNOTECH, Marseille, France	3 3

* preferred antibody based on performance in magnetic cell separations

Tabie 3: Purging Breast Carcinoma Cells (BT20 or T47D cells).

Cell Type	Lineage Depletion	anti-Breast Carcinoma Antibodies	log Tumor Cell Depletion
Previously Frozen Bone Marrow	Purge Only	5E11 5E11, H23A 5E11, 6E7	1.8 3.7, 3.7 3.0
Previously Frozen Bone Marrow	Lineage Depletion and Purge	5E11 RAR BRST1 5E11, H23A 5E11, RAR, BRST1	>5.8, 3.9, 4.7 >5.8, 4.3, 4.7 4.9 >5.2, 4.4 >5.8
Peripheral Blood Leukapheresis	Purge only	5E11 H23A 5E11, H23A	1.9, 1.9 1.7 2.3
Peripheral Blood Leukapheresis	Lineage Depletion and Purge	5E11, H23A	5.6
Fresh Bone Marrow	Lineage Depletion and Purge	5E11, H23A	4.6, 4.4

Table 4: Recovery of Hematopoietic Colony Forming Cells During Lineage Depletion

Colony Assay	% Recovery	
	range	mean
CFU-GM	60-100	75
BFU-E	71-100	92
LTCIC	72->100	100

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Table 5: Enrichment of CAMA Breast Carcinoma Tumor Cells From Blood and Bone Marrow

Exp #	Sample	#CAMA in Start	% CAMA in Start	% CAMA in Flow	% Recovery CAMA	Log Enrich. CAMA
1	BM	1.1/10^2	1.06	91.07	72.41	1.9
2	BM	2.2/10^2	2.18	96.40	44.12	1.6
		2.1/10^3	0.21	82.16	75.00	2.6
		2.1/10^4	0.02	32.01	60.00	3.2
3	BM	2.6/10^3	0.26	62.54	?*	2.4
		2.6/10^4	0.026	11.21	?*	2.6
		2.6/10^5	0.0026	2.01	?*	2.9
		2.6/10^6	0.00026	0.13	?*	2.7
4	PB	1.9/10^3	0.19	86.21	33.33	2.7
		1.9/10^4	0.019	37.06	18.33	3.3
		1.9/10^5	0.0019	7.42	20.00	3.6
		1.9/10^6	0.00019	0.77	19.33	3.6

?* Cell numbers were too low to count accurately.

THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. An antibody composition comprising antibodies specific for glycophorin A, CD3 CD24, CD16, CD14, non-hematopoietic antigens expressed on tumor cells, and optionally one or more antibodies specific for CD45RA, CD38, CD36, CD56, CD2, CD19, CD66e, and CD66b.

2. An antibody composition comprising antibodies specific for glycophorin A, CD3, CD24, CD16, CD14, CD56, CD2, CD19, and CD66b. and optionally antibodies specific for non-hematopoietic antigens.

3. An antibody composition as claimed in claim 1 or 2, wherein the antibodies are monoclonal antibodies.

4. An antibody composition as claimed in claim 1, 2, or 3 wherein the antibodies are labelled with a marker or they are directly or indirectly conjugated to a matrix.

5. An antibody composition as claimed in claim 1, 2, 3, or 4 wherein the antibodies are labelled with biotin or a fluorochrome.

6. An antibody composition as claimed in claim 5 wherein the matrix is magnetic beads, a panning surface, dense particles for density centrifugation, an adsorption column, or an adsorption membrane.

7. An antibody composition as claimed in claim 2 wherein each of the monoclonal antibodies is incorporated in a tetrameric antibody complex which comprises a first monoclonal antibody of a first animal species from the antibody composition as claimed in claim 2, and a second monoclonal antibody of the first animal species which is capable of binding to at least one antigen on the surface of a matrix, which have been

conjugated to form a cyclic tetramer with two monoclonal antibodies of a second animal species directed against the Fc-fragments of the antibodies of the first animal species.

8. An antibody composition as claimed in claim 1, consisting of (a) antibodies specific for glycophorin A, CD3, CD24, CD16, CD14, CD19, CD66b, CD56 and CD2; or (b) antibodies specific for glycophorin A, CD3, CD24, CD16, CD14, CD19, CD66b, CD56, CD2, CD36, CD38, and CD45RA.

9. An antibody composition as claimed in claim 1, consisting of antibodies specific for glycophorin A, CD3, CD24, CD16, CD14, CD19, CD66b, CD56, CD2, CD36, CD38, CD45RA, and for non-hematopoietic antigens expressed on tumor cells.

10. An antibody composition as claimed in claim 9 which contains antibodies specific for antigens on the surface of cells from breast or lung carcinoma, or neuroblastoma.

11. An antibody composition as claimed in claim 10 which comprises D2.10 or 10F7MN, UCHT1, 6F10.3, T199, J4119, MEM15, 3G8 or MEM154, 8OH3 or B13.29, FA6.152 or IVC7, AD2.2, T16, J33, 5E11, H23A, 6E7, RAR, and BRST1.

12. A process for enriching and recovering human hematopoietic progenitor cells and stem cells in a sample containing human hematopoietic stem cells, and tumor cells comprising reacting the sample with an antibody composition containing antibodies capable of binding to the antigens glycophorin A, CD3 CD24, CD16, and CD14, and optionally CD45RA, CD38, CD36, CD56, CD2, CD19, CD66e, and/or CD66b, and antibodies specific for non-hematopoietic antigens expressed on tumor cells, under conditions so that cell conjugates are formed between the antibodies and cells in the sample containing the antigens glycophorin A, CD3 CD24,

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CD16, and CD14, and optionally CD19, CD20, CD56, CD2, CD19, CD66e, and/or CD66b and non-hematopoietic antigens on their surfaces; removing the cell conjugates; and, recovering a cell preparation which is enriched in human hematopoietic progenitor cells and stem cells.

13. A process as claimed in claim 12 wherein the antibody composition consists of antibodies specific for glycophorin A, CD3, CD24, CD16, CD14, CD19, CD66b, CD56, CD2, CD36, CD38, Cd45RA, and for non-hematopoietic antigens expressed on tumor cells.

14. A process as claimed in claim 12 or 13, wherein the antibodies in the antibody composition are monoclonal antibodies.

15. A process as claimed in claim 12, wherein the antibodies in the antibody composition are labelled with a marker or they are conjugated to a matrix.

16. A process as claimed in claim 12, wherein the antibodies in the antibody composition are labelled with biotin or a fluorochrome.

17. A process as claimed in claim 12 wherein the matrix is magnetic beads, a panning surface, dense particles for density centrifugation, an adsorption column, or an adsorption membrane.

18. A process as claimed in claim 14, wherein each of the monoclonal antibodies in the antibody composition is incorporated in a tetrameric antibody complex which comprises a first monoclonal antibody of a first animal species from the antibody composition as claimed in claim 2, and a second monoclonal antibody of the first animal species which is capable of binding to at least one antigen on the surface of a matrix, which have been conjugated to form a cyclic tetramer with two monoclonal antibodies of a

second animal species directed against the Fc-fragments of the antibodies of the first animal species.

19. A process as claimed in claim 12 wherein the tumor cells are from a breast or lung carcinoma, or neuroblastoma, and the antibody composition contains antibodies specific for antigens expressed on the surface of cells from breast or lung carcinoma, or neuroblastoma.

20. A process as claimed in claim 19 wherein the tumor cells are from a breast carcinoma, and the antibody composition contains one or more of the antibodies designated 5E11, H23A, 6E7, RAR, and BRST1.

21. A kit useful in performing the process as claimed in claim 12 comprising antibodies specific for glycophorin A, CD3, CD24, CD16, CD14, and for non-hematopoietic antigens expressed on tumor cells, and optionally CD45RA, CD38, CD36, CD38, CD56, CD2, CD19, CD66e, CD66b, and instructions for performing the process.

22. Cell compositions obtained in accordance with the process as claimed in claim 12.

23. Use of an antibody composition as claimed in claim 1 in a negative selection technique to recover cell preparations enriched in human hematopoietic progenitor and stem cells.

24. An antibody composition comprising antibodies specific for glycophorin A, CD3, CD19, CD36, CD14, CD16, CD66b, CD56, CD38, CD36, CD45, and optionally CD41, CD33, CD20, CD22, CD29, CD2, CD45RA, and/or CD10.

25. An antibody composition as claimed in claim 24, wherein the antibodies are monoclonal antibodies.

26. An antibody composition as claimed in claim 25 wherein the antibodies are labelled with a marker or they are directly or indirectly conjugated to a matrix.

27. An antibody composition as claimed in claim 26 wherein the antibodies are labelled with biotin or a fluorochrome.

28. An antibody composition as claimed in claim 26 wherein the matrix is magnetic beads, a panning surface, dense particles for density centrifugation, an adsorption column, or an adsorption membrane.

29. An antibody composition as claimed in claim 26 wherein each of the monoclonal antibodies is incorporated in a tetrameric antibody complex which comprises a first monoclonal antibody of a first animal species from the antibody composition as claimed in claim 25, and a second monoclonal antibody of the first animal species which is capable of binding to at least one antigen on the surface of a mati which have been conjugated to form a cyclic tetramer with two monoclonal antibodies of a second animal species directed against the Fc-fragments of the antibodies of the first animal species.

30. A process for enriching for non-hematopoietic metastatic tumor cells in a sample containing the tumor cells and hematopoietic cells comprising

(a) reacting the sample with an antibody composition comprising antibodies specific for glycophorin A, CD3, CD19, CD36, CD14, CD16, CD66b, CD56, CD38, CD36, CD45, and optionally CD41, CD33, CD20, CD22, CD29, CD2, CD45RA, and/or CD10. under conditions so that conjugates are formed between the antibodies and hematopoietic cells in the sample expressing the antigens glycophorin A, CD3, CD19, CD36, CD14, CD16, CD66b, CD56, CD38,

CD36, CD45, and optionally CD41, CD33, CD20, CD22, CD29, CD2, CD45RA, and/or CD10;

(b) removing the cell conjugates, and

(c) recovering a cell preparation enriched in the tumor cells.

31. A process for detecting non-hematopoietic metastatic tumor cells in a sample comprising

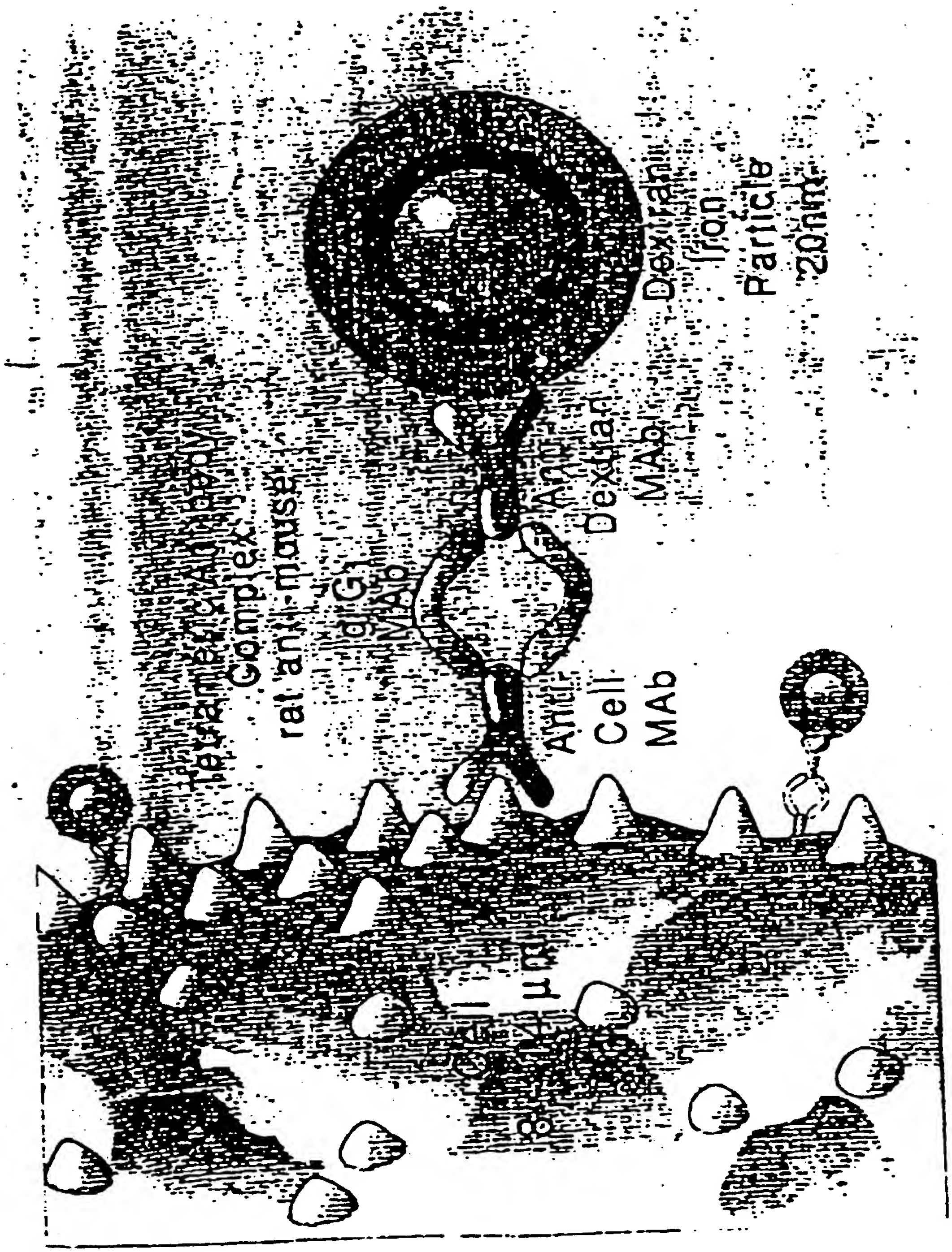
(a) reacting the sample with an antibody composition comprising antibodies specific for glycophorin A, CD3, CD19, CD36, CD14, CD16, CD66b, CD56, CD38, CD36, CD45, and optionally CD41, CD33, CD20, CD22, CD29, CD2, CD45RA, and/or CD10 under conditions so that conjugates are formed between the antibodies and hematopoietic cells in the sample expressing the antigens glycophorin A, CD3, CD19, CD36, CD14, CD16, CD66b, CD56, CD38, CD36, CD45, and optionally CD41, CD33, CD20, CD22, CD29, CD2, CD45RA, and/or CD10;

(b) removing the cell conjugates; and

(c) recovering a cell preparation enriched in the tumor cells.

32. A kit useful in performing a process as claimed in claim 30 or 31 comprising antibodies specific for glycophorin A, CD3, CD19, CD36, CD14, CD16, CD66b, CD56, CD38, CD36, CD45, and optionally CD41, CD33, CD20, CD22, CD29, CD2, CD45RA, and/or CD10, and instructions for performing the process as claimed in claim 29 or 30.

FIGURE 1


Complex
rat anti-mouse
MAb
Anti-Dextran MAb
Dextran Iron Particle 20nm
Anti-Cell MAb